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Use of an Inert Radioactive Particle for Measuring Particle Accumulation by Filter-Feeding Bivalve Molluscs

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Abstract

The use of an inert, radioactively labeled microsphere as a measure of particle accumulation (filtration activity) by *Mulinia lateralis* (Say) and *Mytilus edulis* L. was evaluated. Bottom sediment plus temperature and salinity of the water were varied to induce changes in filtration activity and thus enable us to judge the effectiveness of the particles as filtration indicators. The rate of loss of radioactivity observed in *Mulinia lateralis* held for several days after exposure confirmed that the particles are ingested by the organisms. The observed differences in particle accumulation and the ease of administration and measurement combine to make the use of these microspheres a valuable tool in studies dealing with particle distribution and accumulation.

Introduction

The rate of water transport and efficiency of particle retention by the gills of bivalve molluscs has long been of interest to biologists. Many of the studies concerning mechanisms of pumping and factors affecting pumping rate and filtration efficiency have been documented by Jørgensen (1966, 1975). A common area in nearly all these studies is the methodology employed to measure pumping and filtration rates.

Basically there are two methods for measuring water transport by filter-feeding bivalves. The direct method requires physical separation of the inhalent and exhalent currents, thus permitting measurements of water volumes pumped and size and number of particles retained by the gills. In many bivalves, however, the morphology of the organism prohibits use of the direct method and an indirect approach is utilized. This indirect method estimates pumping rates by quantifying the rate of particle removal from the water as measured by changes in optical density or actual particle counts.

Aside from the physical difficulty of separating the two siphons, the constraints imposed on the organism by the experimental apparatus of the direct method make such data of questionable value for application to natural conditions (Jørgensen, 1975). The indirect method partly overcomes this limitation, but presents others. Three basic problems include: (a) inability to measure filtration efficiency, hence one must assume 100% efficiency or report the results as minimal pumping rates (Ward and Aiello, 1973); (b) the possible introduction of particulate matter by the test organism in the size range of the indicator particles which interferes with counting (Vahl, 1972); and (c) allowances for the natural division of cells must be made when microalgae are the indicator particles.

There have been a number of studies using radioactively labeled particles (Chipman and Hopkins, 1954; Blake, 1961; Allen, 1962; McMahon and Rigler, 1965). All these studies used labeled microalgae as the indicator particle. Jones *et al.* (1974) used encapsulated ^{14}C-labeled starch particles to show assimilation by *Artemia* sp. The coating in this case was to maintain the food particles in the proper size range and reduce bacterial contamination. Measuring radioactivity avoids many problems of particle counting, but care must be taken to assure that the radioactivity measured is due to that within the particle and not its presence in the water.

Our studies have led us to try to relate the filtering activity of several bivalve molluscs in response to various environmental factors and toxic materials. The problems with the materials and methods listed above led us to seek a radio-tracer technique but using a particle that would: (a) bond with a radioactive label, (b) not leach the label when suspended in seawater, (c) be nondivisible and nondegradable, and (d) be of a size and consistency that would permit removal from the water by filter-feeding organisms. Since it would often be desirable to measure the radioactivity (particle accumulation) in the organism, the label should be a gamma-emitter.

In preliminary work, radioactive cadmium (^{109}Cd) was bonded to materials such as reduced bottom sediments, sulfide-treated diatomaceous earth, ion-exchange resins (Chelex 100) and molecular seives (Sephadex C25, G10 and G100). All these proved unsatisfactory as indicator particles as the leaching rate of radioactivity was excessive when dispersed in seawater. Another approach was to label a unicellular algae (*Isochrysis galbana*) with ^{109}Cd and use this as an indicator particle. The chief disadvantages of this method were cell division and the possibility of cell lysing, thus causing the release of radioactive material to the water. The only product that appeared to meet the prescribed criteria was a commercially available prelabeled preparation called Tracer Microspheres[1] produced by Nuclear Products of the 3M Company. These are small, spherical particles which have been labeled with one of a variety of isotopes. The microsphere, as described by the manufacturer, is composed of carbonized plastic (polystyrene) and a trace amount of the desired nuclide. The nuclide is not coated on but incorporated within the particle which effectively eliminates tracer leaching in the suspension medium. The particles have an absolute density of approximately 1.3 g/cm^3.

The purpose of this paper is to report on an evaluation of the suitability of these microspheres as indicators of filtration activity as measured by particle accumulation in two species of bivalve molluscs. The test organisms, *Mulinia lateralis* (Say) and *Mytilus edulis* L., were subjected to a number of single-variable experiments designed to induce changes in filtration activity. The experimental variables were: temperature, salinity and sediment type. The experiments were performed principally to determine the effectiveness of the microspheres as a tool for quantifying a change in pumping/filtering activity.

[1] Mention of specific products herein does not constitute endorsement by the U.S. Environmental Protection Agency.

Materials and Methods

The *Mulinia lateralis* were collected all together in Narragansett Bay, Rhode Island, and maintained in a sand substrate in flowing seawater. The *Mytilus edulis* were collected 48 h prior to testing and allowed to attach with byssal threads to circular plastic dishes 9 cm in diameter. Shell lengths ranged from 8 to 14 mm for *Mulinia lateralis* and from 12 to 23 mm for *Mytilus edulis*. Water temperature at the time of collection ranged from 9° to 16°C, depending on the time of year. Salinity varied from 28 to 32‰. The molluscs were held in flowing water at ambient temperature and salinity conditions until 24 h prior to testing. At this time they were removed and placed in test chambers containing water maintained at the desired conditions.

The experimental chambers were 4 l wide-mouth jars containing 2 l of filtered seawater. There were two replicates of each test condition, each containing at least 10 specimens. The microspheres used in this study were 15 ± 5 µ (manufacturer's measurements) in diameter, and were labeled with ^{141}Ce, half-life 32.5 days.

One hundred microliters of the microsphere stock suspension was transferred to a number of test tubes equal to the number of treatments. The radioactivity of each tube was counted in a Packard Liquid Scintillation Counter adapted for measuring gamma activity (Jackim, 1975). The volumes of the microsphere suspension were adjusted and recounted until the radioactivity levels in all tubes were uniform. Adjustment volumes were usually less than 10 µ. Samples with radioactivity levels adjusted to within 2% of each other were found to be equivalent in particle concentration. The microspheres were then added to the test chambers by multiple washings and vigorously mixed with a stainless-steel stirring rod to assure uniform distribution. The radioactivity in the test chambers decreased from 21,000 to 12,000 cpm during the 1½ months of testing due to the natural decay of the isotope, but particle concentrations remained constant (50 ± 5 particles/ml) within and between experimental periods. This particle concentration was believed low enough not to affect filtration activity (Winter, 1973),

Table 1. *Mulinia lateralis* and *Mytilus edulis*. Relative particle accumulation as affected by temperature, salinity and substrate. Units of measure expressed as number of particles per shell length. Any pair or set of means not underscored by same line differs significantly at 0.05 level. nd: no data

Temperature		5°C	10°C	15°C	20°C	25°C
Mulinia lateralis	$\bar{X}$	11.50	23.59	27.31	144.96	49.39
	σ	10.50	26.16	41.95	72.62	36.08
Mytilus edulis	$\bar{X}$	106.96	126.34	100.11	130.15	122.81
	σ	62.82	34.55	47.41	50.96	36.80

Salinity 30‰, no sediment

Salinity		10‰	15‰	20‰	25‰	30‰
Mulinia lateralis	$\bar{X}$	nd	53.04	124.87	100.81	121.30
	σ	nd	48.97	79.94	57.75	69.85
Mytilus edulis	$\bar{X}$	5.03	40.95	103.24	123.56	109.75
	σ	6.00	59.06	62.13	35.14	55.78

Temperature 20°C, no sediment

Sediment type		No sediment	Sand	Mud
Mulinia lateralis	$\bar{X}$	104.35	74.77	35.72
	σ	87.79	52.15	53.96

Temperature 20°C, salinity 30‰

but sufficient to be easily detectable in the test water and organisms.

The jars were maintained at the desired experimental conditions in temperature-controlled (5°, 10°, 15°, 20° and 25°C ± 0.5 C°) environmental chambers. *Mulinia lateralis* were placed in jars with or without sediment, as test conditions dictated. *Mytilus edulis* were suspended in the jars still attached to the plastic plates to avoid breaking byssal thread attachment. The organisms were allowed to filter the test water for 2 h, removed, rinsed in filtered seawater, placed in individual plastic test tubes, and gamma activity was recorded for 2 min. Following several of the experiments the test water was filtered through a 0.45 µ Millipore filter and the filtrate and filter pad measured for gamma activity. Data are expressed as accumulated radioactivity per shell length in order to compensate for size differences among test specimens. The number of particles per organism is calculated by dividing the measured radioactivity by the activity (cpm) per particle. The means and standard deviations were calculated for each treatment (Table 1). Reported differences in particle accumulation are significant at the 95% confidence interval level as analyzed with the Duncan's New Multiple Range Test (Steele and Torrie, 1960). An estimation of the amount of shielding afforded by the shell was ascertained by counting radioactivity before and after shucking.

Results

A 2 l seawater volume and a 2 h exposure period was sufficient to allow *Mulinia lateralis* and *Mytilus edulis* to accumulate easily measurable amounts of the microspheres without significantly reducing particle concentration in the water. Particle reduction in any treatment never exceeded 15% of the original concentration. Use of other experimental parameters or other study species which may differ in behavior or size could necessitate variations in water volume and/or test duration.

No measurable gamma activity was found in the test water following filtration, thus supporting the manufacturer's claim of non-leaching of the label. Measurements of vertical distribution of radioactivity in the test containers following mixing indicated that the particles slowly settle toward the

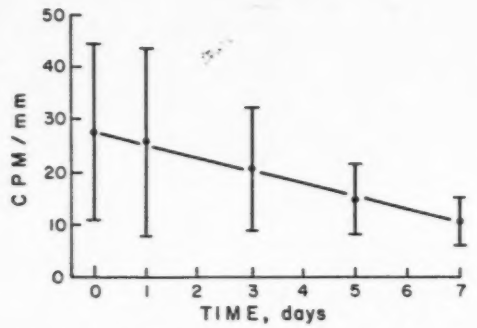

Fig. 1. *Mulinia lateralis*. Rate of microsphere elimination following 2 h uptake period. Molluscs exposed at 20°C, 30‰; elimination at 16°C, 29‰

bottom. No determination of settling rates or the amount of turbulence required to maintain the particles in the water column was made.

Shielding of radioactivity by the shell of *Mytilus edulis* was negligible. The mean radioactivity of 10 molluscs counted whole was 170 cpm. The mean radioactivity of the soft body parts of the same 10 specimens was 161 cpm, a reduction of 5.3%. This reduction in radioactivity is most probably attributable to particles associated with the shell and water in the mantle cavity.

The rate of particle elimination by *Mulinia lateralis* when held in flowing, nonfiltered seawater (16°C), following the standard 2 h exposure to the microsphere suspension is shown in Fig. 1. The 5 to 6 days required for a 50% reduction in animal radioactivity indicates that the majority of the particles were being accumulated in the digestive tract and not simply being trapped on the gills.

Discussion

It is evident that the use of this material and technique allows rapid, easy and accurate measurements of filtration activity. The degree of shielding of radioactivity by the shell was minimal in *Mulinia lateralis* and *Mytilus edulis* in this study, and thus no correction was necessary. In studies comparing organisms of widely differing sizes, two or more species and/or use of a lower energy isotope may make the application of this correction factor appropriate.

The advantage of nondestructive counting is obvious. Aside from being much easier and faster than shucking the molluscs, the results indicate that more reliable values, at least for *Mytilus edulis*, can be obtained when the organism is counted in this manner. A very real problem that nondestructive counting presents, using scintillation counting, is that it severely limits the size of organisms that can be used.

Although the use of microspheres does not eliminate the inherent problems of measuring filtration efficiency by the indirect method, it does: (a) avoid the problem of cell division when using microalgae, (b) eliminate the problem of masking the indicator particle by bivalve-expelled material, (c) allow measurements of particle accumulation by counting either the organism or the water. The greatest advantage of Tracer Microspheres as indicator particle is that they eliminate the possibility of loss of the radioactive label to the water since the nuclide is incorporated in the particle. All the other materials tested released radioactive material which, by the very nature of the test, made accurate particle-accumulation determinations impossible. This material also offers advantages over the use of labeled microalgae as there is no lengthy incubation period, no possibility of cell lysing, or no dilution by cell division. Also, the use of various-sized particles each with its own label offers interesting possibilities for studies involving such factors as particle-size selectivity and variations in filtration efficiency.

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